Corrosion of Machined Titanium Dental Implants Under Inflammatory Conditions

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Abstract: The effects of hyperglycemia, altered cell function, or inflammatory mediators on implant corrosion are not well studied; yet, these effects are critical to implant biocompatibility and osseointegration. Because implant placement is burgeoning, patients with medically compromising systemic conditions such as diabetes are increasingly receiving implants, and the role of other inflammatory diseases on implant corrosion also needs investigation. In the current study, the corrosion properties of commercially available, machined titanium implants were studied in blood, cultures of monocytic cells, and solutions containing elevated dextrose concentrations. Implant corrosion was estimated by open circuit potentials, linear polarization resistance, and electrical impedance spectroscopy (EIS) for 26 h. In selected samples, THP1 monocytic cells were activated for 2 h with Lipopolysaccharide prior to implant exposure, and IL-1β secretion was measured to assess the affect of the implants on monocyte activation. Implants under conditions of inflammatory stress exhibited more negative E_{corr} values, suggesting an increased potential for corrosion. Linear polarization measurements detected increased corrosion rates in the presence of elevated dextrose conditions over PBS conditions. EIS measurements suggested that implants underwent surface passivation reactions that may have limited corrosion over the short term of this test. This result was supported by cyclic polarization tests. IL-1β secretion was not altered under conditions of corrosion or implant exposure. The results suggest that inflammatory stress and hyperglycemia may increase the corrosion of dental endosseous titanium-based implants, but that longer, more aggressive electrochemical conditions may be necessary to fully assess these effects. © 2008 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 88B: 474–481, 2009

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INTRODUCTION

Endosseous dental implants are used to replace missing teeth and retain or support dental prostheses. The placement of endosseous implants is increasing in dentistry[1] and between 300,000 and 400,000 implants are placed each year in the U.S. alone.[1] The number of implants placed is projected to increase 12–15% per year.[2] Although these implants are placed in all demographic groups, the elderly (>65 years old) receive the greatest number of implants because of an increased incidence of edentulism in this age group.[3,4]

Endossesous dental implant placement is remarkably successful, with a published 5-year success rate of 98.6%.[5] However, this high rate of success belies problems that may occur when implants are placed in clinical environments that are less than ideal. Even when clinical bone conditions suggest a favorable prognosis, some subpopulations are at much greater risk for implant failure.[6] For example, patients who smoke tobacco have a 15% failure rate,[5] and those who have radiation therapy to the head and neck have a 64% failure rate.[7,8] Invasive dental procedures such as endossesous implant placement are contraindicated for patients on intravenous bisphosphonate therapy due to the risk of osteonecrosis.[9] Type I and II diabetes also significantly increases the risk of implant failure.[10,11] The risk to diabetic patients is particularly disconcerting because of the growing incidence of this disease,[12,13] the number of undiagnosed diabetics,[12,13] and the high incidence of type II diabetes in the elderly.[12–14]

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The physiological factors of diabetes that contribute to the failure of endosseous dental implants are not known. However, some evidence links microvascular changes and impeded wound healing responses as mediators of failed osseointegration in the weeks after implants are surgically placed.[11,15] Animal models have confirmed that when diabetic conditions are simulated, osseointegration is impeded.[11] Studies suggest that inflammation (acute or chronic) contributes to the failure of dental implants.[16,17]

Corrosion of endosseous implants is a primary variable in the response of bone and other periodontal tissues to implant placement.[18–20] Several studies have used a variety of electrochemical techniques to measure the corrosion properties of implant materials and have documented the corrosion of many alloys in biological environments. Protein adsorption alters corrosion depending on pH, the type of alloy, surface condition of the alloy, the presence of other metal ions, and the nature of protein adsorption onto the alloy.[21–28] Other studies have assessed the effects of hydrogen peroxide,[29–31] proteins,[22-24,26,28,32] mechanical stress,[33–36] wear/fretting,[37,38] and cells[31,33,34,39-42] on implant material corrosion properties and have used animal models to correlate metal ions release to *in vitro* corrosion testing.[43,44] However, these studies generally have been restricted to the use of flat or cylindrical "bulk" samples in commercial electrolytes (i.e., saline, serum, or artificial saliva).[19,38,42,45–50] The native geometry of endosseous implants is complex and diverse and may influence implant corrosion properties. This complex geometry, the oxide layers on implant surfaces, and any damage to these oxides during implant placement may synergize to change corrosion properties of the implant and therefore biological response. Furthermore, commercial electrolytes do not model many aspects of clinical conditions, such as the occurrence of cellular elements, inflammatory activators, or high glucose environments. Therefore, the electrochemical properties of commercial implants under circumstances of disease and inflammation that are increasingly common in elderly patients are relatively poorly studied.

The current study tested the hypothesis that the electrochemical corrosion properties of endosseous, titanium-based implants will differ as corrosion environments are adapted to simulate common clinical conditions and diseases. This investigation used common commercial endosseous implants to characterize the corrosion in environments with blood, inflammatory cells, and elevated glucose.

MATERIALS AND METHODS

Implant Materials

Nobel Biocare Branemark Mark II machined commercially pure (grade 2, Göteborg, Sweden) titanium clinical dental implants were used in this study. The implants measured 4.3–5.5 mm in diameter and varied in length from 8 to 13 mm. These implants were exposed to the following solutions: phosphate-buffered saline (PBS), cell-culture media with monocytic cells, cell-culture media with monocytic cells and lipopolysaccharide (LPS, *E.coli*, serotype 026:B6, Sigma, 1 μg/mL, 2 h), cell-culture media with monocytic cells and dextrose (15 m*M*), cell-culture media with monocytic cells, LPS, and dextrose and reconstituted packed red blood cells (20% fetal bovine serum and PBS) with monocytic cells. All implants were received in the original manufacturer's packaging and opened under sterile conditions.

Cell-Culture

The monocyte was chosen as a focus for the current investigation because monocytic cells play a significant role in inflammation. THP1 human monocytes (ATCC TIB 202) were chosen to model primary monocytes because they are of human origin, well characterized,[51] and exhibit cytokine secretion in response to activators such as LPS.[39–41] THP1 monocytes were cultured in RPMI plus 10% fetal bovine serum. In specific experiments, THP1 monocytes were cultured in media supplemented with 15 m*M* dextrose or activated using LPS. LPS was chosen because it is an important mediator of inflammation and has been shown to circulate in smokers and patients with periodontal disease.[42,43,52–54] Dextrose (15 m*M*) was chosen because it is equivalent to a blood sugar level of 270 mg % which is common in for an uncontrolled diabetic or a patient with undiagnosed type II diabetes.

Corrosion Tests

The electrochemical setup consisted of a standard 3-electrode microcell (Perkin Elmer), a computer-controlled potentiostat (Perkin Elmer Potentistat/Galvaniostat 273A) standardized per ASTM G5 (ASTM G5: Standard Reference Test Method for Making Potentiostatic and Potentiodynamic Anodic Polarization Measurements), stirrer plate with stirrer bar, titanium implant as the working electrode, Ag/AgCl (with saturated potassium chloride) reference electrode, and platinum wire-mesh as the counter electrode. The exposed working electrode surface area was $\sim$1.5 cm^2 (based on Noble Biocare data) and the electrolyte volume was 100 mL. The THP1 cells were maintained in suspension by gently stirring the electrolyte solution. The open-circuit potential (OCP) of the sample was allowed to stabilize (less than a 0.2 mV/s change) for 2 h. The linear polarization resistance (LPR) was performed by scanning through a potential range ($\pm$20 mV) close to the corrosion potential and was measured at 2 h, and again at 10, 18, and 26 h. For the LPR technique, the polarization resistance is the slope of the current versus potential line and is used to calculate the corrosion rate [i_{corr} in A/cm^2] using the Princeton Applied Research Corrosion Software (Softcorr III) and the corrosion rate in millimeters per year (mmpy) reported. Following the last LPR measurement, electrolyte samples (1–2 mL) were removed from the microcell before electrochemical testing continued in the remaining 98 mL

of solution. After the pH of the electrolyte sample was measured, the samples were centrifuged at 1000*g* for 5 min, the supernatant removed from the cell pellet and each frozen at −20°C for further analysis. All data were statistically compared using ANOVA and Tukey post-hoc analysis ($\alpha = 0.05$).

After the electrolyte sample was removed and resting potential reestablished, electrochemical impedance spectroscopy (EIS) was used to evaluate the corrosion mechanism of the implants and the characteristics of any coating that may have formed. Alternating current amplitude of 10 mV over a frequency range of 100 kHz to 100 mHz was applied while the responding current and potential were recorded. These values were used to determine the real (Z') and imaginary (Z'') components of the impedance, which were plotted with a Nyquist plot, or the total impedance (|Z|) and phase angle, which were seen in a Bode plot as a function of the frequency.

Tafel and cyclic polarization curves were determined in similar but separate experiments with respect to the reference electrode. The OCP of the sample was allowed to stabilize (less than a 0.2 mV/s change) for 2 h. Cathodic tafel polarization curves were generated using a potential ramp of 0.17 mV/s from −250 to 25 mV from OCP. A minimal anodic potential of 25 mV was used due to the passivation behavior of the titanium. Anodic and cathodic Tafel slope values were calculated using the Princeton Applied Research Corrosion Software-SoftCorr III using the slope of the current versus potential line and calculating the corrosion rate (i_{corr} in A/cm^2) from the Stern-Geary equation. Then corrosion rate in millimeters per year (mmPY), was calculated from the corrosion current using Faraday's law equation. Cyclic polarization curves were similarly determined using a potential ramp of 0.17 mV/s from 500 mV below OCP to a vertex potential of 750 mV over OCP and a final potential of 0 V. Only one implant was used per test solution due to the destructive nature of the cyclic polarization experiments and the cost of the implants.

Morphological Analysis

Implants were photographed at magnifications ranging from 5× to 100× after each test. Prior to microscopy, the implants were fixed in 4% formalin and stained with trypan blue to allow visualization of the cells.

Cytokine Secretion

IL-1β is produced primarily by monocytes in response to an inflammatory stimulus. Conditioned media from THP-1 monocytes were collected before and after corrosion testing and then assayed for secreted cytokines. IL-1β was measured by means of ELISA (R&D Systems), following manufacturer's directions, and employing controls with medium alone and medium with cells but no activator (LPS or implant or electrochemical test). Detection limit for the IL-1β ELISA kit was 0.057 pg/mL. Conditions were statistically compared using ANOVA and Tukey post-hoc analysis ($\alpha = 0.05$).

RESULTS

E_{corr} values were more electronegative in biological solutions than in PBS and were significantly different at 10 and 18 h but not at 2 and 26 h (letter groupings, Figure 1, upper, ANOVA, Tukey, $\alpha = 0.05$). Although E_{corr} values became more positive with time, the open circuit potentials did not statistically differ over the 26-h test period in any tested electrolyte (ANOVA, Tukey, $\alpha = 0.05$, Figure 1, upper) and therefore were grouped to obtain more statistical power to see differences among different corrosion conditions. When the data were combined, differences in E_{corr} were more evident among the electrolytes (Figure 1, bottom). Interestingly, the open circuit potentials for all inflammatory and diabetic conditions were significantly more electronegative than those for PBS and cell-culture media with THP1 cells.

The corrosion rates of the implants in biological solutions were significantly higher than in PBS at 10 and 18 h but not at 2 and 26 h (Figure 2, upper, ANOVA, Tukey, $\alpha = 0.05$). As with the E_{corr} values, the corrosion rates moved toward higher values with time but were not statistically different over the 26-h test period in any tested electrolyte (ANOVA, Tukey, $\alpha = 0.05$, Figure 2, upper) and therefore were consolidated. This strategy confirmed the trend that corrosion conditions that contained dextrose or inflammatory conditions caused higher corrosion rates than PBS and were significantly different from the media only conditions (ANOVA, Tukey, $\alpha = 0.05$, Figure 2, lower). Corrosion rates in the presence of cells with or without LPS were also elevated above PBS, but were not statistically distinct from either dextrose-containing conditions or PBS. Corrosion rates in blood were similar to those of THP1 ± LPS.

The EIS spectra showed that under all conditions the titanium implant was passive with no polarization resistance values evident at low frequency. At high frequency, a solution resistance value of 20 Ω was measured for all experiments except those with blood. The environment of blood and THP1 cells showed that two time constants were present with a high frequency of 80 Ω and a low frequency resistance of 3.3E5 Ω. Although the values were shifted, this type of biphasic time constant was seen in all conditions with THP1 cells present in the Bode phase plot (Figure 3, left). However, this biphasic characteristic is not obvious in the absolute impedance Bode plots (Figure 3, right). The two time constants may be associated with the charge transfer reaction and the presence of a protein coating.

The passive corrosion properties of the machined titanium implants also can be seen in the cyclic polarization data (Figure 4). From the negative hysteresis, it is clear that the alloy is not susceptible to either pitting or crevice corrosion in any tested electrolyte (Figure 4, dotted arrow).

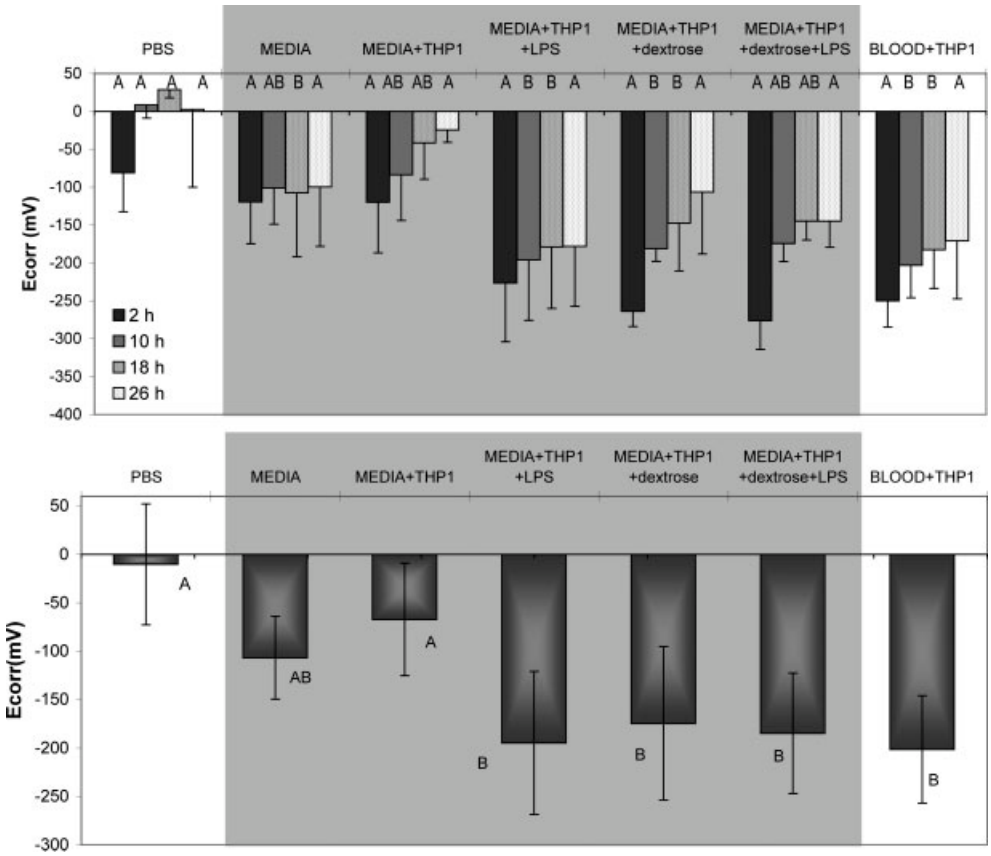

Figure 1. Resting potential (mV, upper) and all time points averaged (mV, bottom) at 2, 10, 18, and 26 h in various electrolytes. Gray-shaded areas highlight conditions with media. Letters indicate significant differences at each time point (upper; for example 2 h data points were compared for all electrolytes, $\alpha = 0.05$) and differences between electrolytes (lower; $\alpha = 0.05$). The resting potential of the implants drifted more electropositive over time. Error bars indicate one standard deviation ($n = 3$).

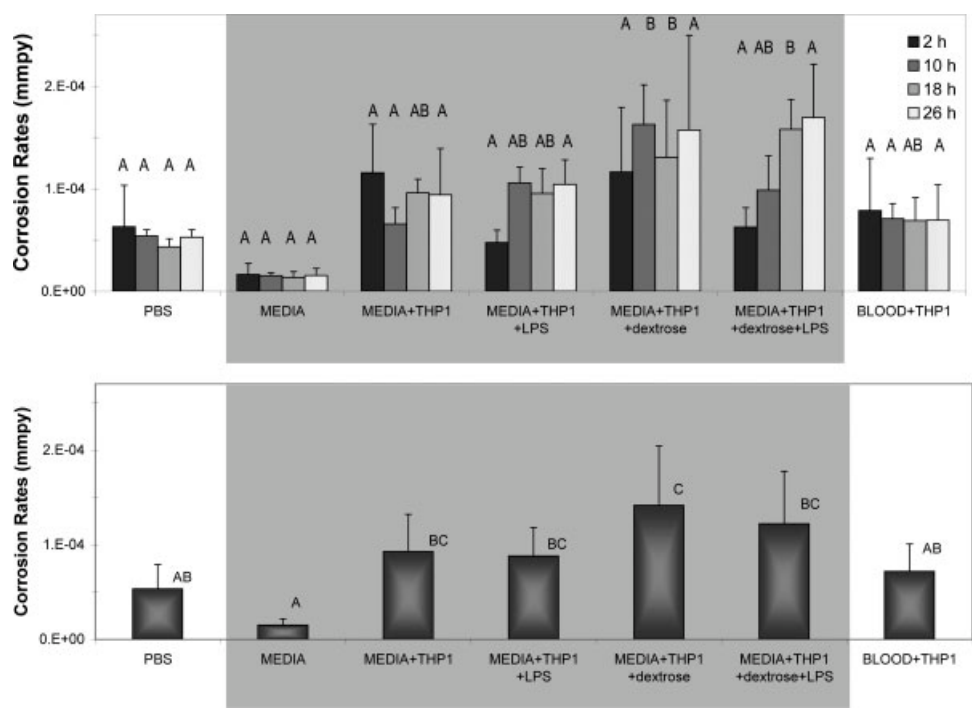

Figure 2. Corrosion rates (mmpy, upper) and all time points averaged (mmpy, lower) at 2, 10, 18, and 26 h in various electrolytes. Gray-shaded areas highlight conditions with media. Letters indicate significant differences at each time point (upper; for example 2 h data points were compared for all electrolytes, $\alpha = 0.05$) and differences between electrolytes (lower; $\alpha = 0.05$). Error bars indicate one standard deviation ($n = 3$).

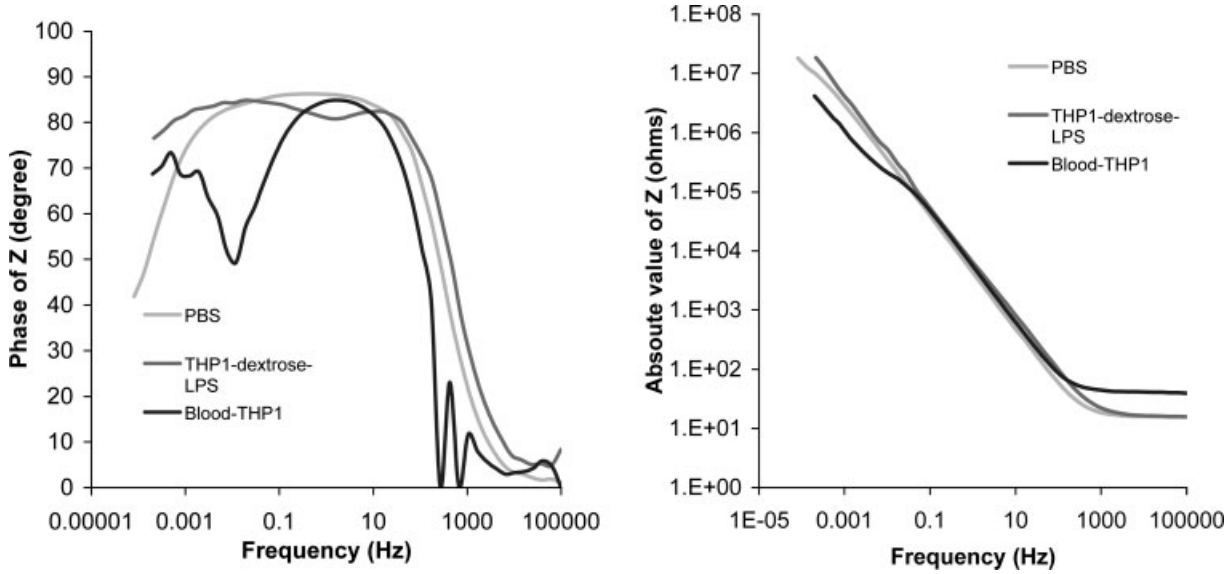

Figure 3. Representative EIS curves for implants in PBS, media with THP1 cells, and human blood with THP1 cells. Left: Bode plot: phase of impedance versus log frequency. Right: Bode plot: absolute value of impedance versus log frequency.

All implants except for the implant in PBS contained a passive stabilization region (Figure 4, solid arrow) suggesting the development of a protein coating and is consistent with the biphasic Bode phase plot (Figure 3, right).

The testing environments did not affect the Tafel cathodic polarization results in this passive titanium system (Table I, 105.3-133.8 mV/decade) suggesting that the electrolytes or proteins did not limit the oxygen access to the surface and therefore alter current density. The resting potentials for the PBS and media with THP1 cells were higher during the potentiodynamic testing than the other testing methods (Figure 4). Although it appears that the resting potential determined from the cyclic polarization test looks different for the PBS and media with THP1 cells conditions, these values were for a single experiment. Furthermore, the resting potentials were not significantly different between testing methods for any electrolyte condition.

It should also be noted that the pH of the electrolytes before testing was not significantly different from the pH of the solution after testing. The pH ranged from 7.22 to 7.45 for all tests.

As expected LPS treatment enhanced THP1 cell secretion of IL-1β compared with unactivated THP1 cells. The presence of the implant did not alter IL-1β secretion for any test condition. Furthermore, the IL-1β secretion in the electrolyte removed from the corrosion mini-cell was not

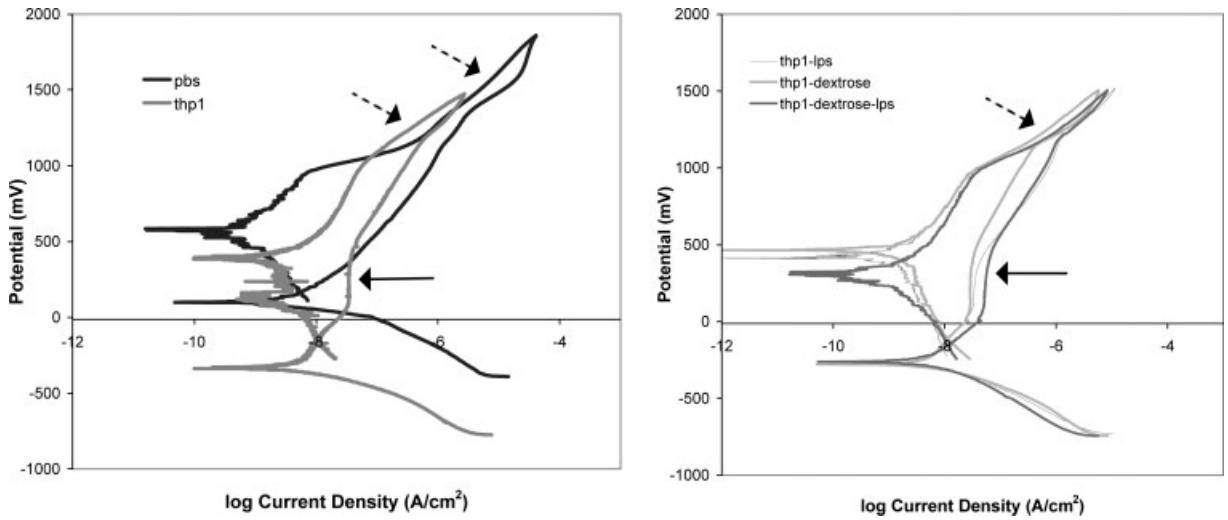

Figure 4. Cyclic polarization curves of implants in various electrolytes (−500 to 750 mV vs. open circuit potential). All implants underwent a negative hysteresis indicating the passive characteristic of titanium (dotted arrow). All implants except the implant in PBS contained a passive stabilization region (solid arrow) suggesting the development of a protein coating.

TABLE I. Tafel Slope and Resting Potential Values for Implants in Various Electrolytes During Potentiodynamic Testing

	β-Anodic Slope (mV/decade)	β-Cathodic Slope (mV/decade)	E_{corr} (mV)
PBS	44.9	105.3	109.7
Media-THP1	18.9	128.4	−216.1
Media-THP1-LPS	24.5	113.7	−181.9
Media-THP1-Dextrose	27.5	126.4	−196.4

significantly different from the control samples (data not shown).

Micrographs

No visible signs of corrosion were evident on the implants (Figure 5). However, cells were seen on the surface of the implants for all linear polarization/EIS tests containing THP1 cells and "clotting" was present on the implants tested in blood.

DISCUSSION

The electrochemical analyses of titanium-based implants suggest that conditions associated with tissue inflammation enhance the thermodynamic tendency of these implants to corrode. Increasingly electropositive E_{corr} values with time suggested that implant surfaces became passivated (Figure 1), as would be expected with titanium-based systems, even though the corrosion rates increased as well. E_{corr} and LPR results both suggested higher corrosion under conditions with activated monocytes or high dextrose levels (Figures 1 and 2). Specifically, E_{corr} values suggested that the presence of LPS or dextrose enhanced the thermodynamic corrosion potential of the implants (by making E_{corr} more negative, Figure 1), yet the LPR measurements did not

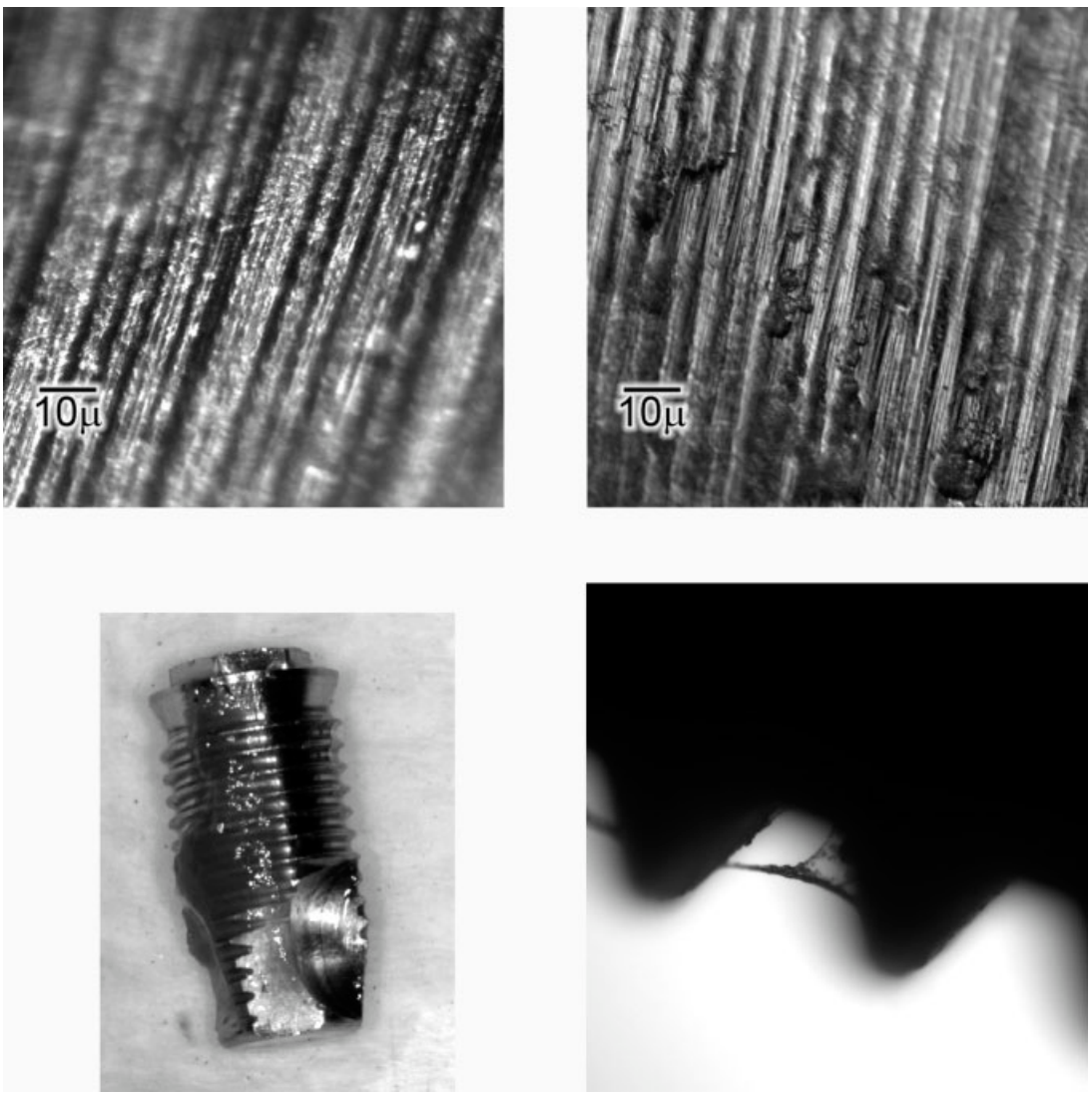

Figure 5. Magnification micrographs. Top left: Implant after corrosion (×10 magnification in PBS). Top right: Implant after corrosion (×10 maginification in media with THP1 cells) shows cells on implant surface. Lower left: Implant with "clot" after corrosion testing in blood. Lower right: Profile of implant with "clot" after corrosion testing in blood.

always indicate significantly higher corrosion rates from the PBS controls (Figure 2). This discrepancy may have stemmed from the relatively short time of the LPR corrosion tests in the current study; increased corrosion rates may occur at times after 24 h. This possibility deserves further study because increasing E_{corr} values suggest a thermodynamic tendency toward higher corrosion.

Many studies have reported that proteins may increase or decrease or do not affect the corrosion rates of various materials.[21-28,32-34,37,39,40,42,50] The interaction between materials and proteins, cells, or their by-products is dependent on several factors including surface charge, roughness, and composition of the material as well as shape, charge, and binding affinities of the molecule.[50] Proteins may increase corrosion by proteins binding and carrying away metal ions from the alloy surface, encouraging further corrosion; proteins limiting oxygen at the surface do discourage passivation as the oxide layer forms or when it is disturbed.[32,37] In the current study, the corrosion rate of the implant was significantly higher under conditions with media containing cells, inflammatory and higher dextrose concentrations than the media only but not significantly different for PBS. It is possible that from corrosion the proteins from the media help protect the implant surface while the cells, activated cells, and higher dextrose concentrations increase the corrosion rate by one of these methods. Again, this possibility deserves further study because increasing the length of the experiment may allow further differences to be observed between the conditions.

In this study, corrosion conditions in human blood were largely equivalent to those with THP1 cells with or without the addition of LPS. E_{corr} measurements (Figure 1) suggested that the corrosion potential in blood was as great as in conditions of dextrose and LPS. LPR measurements (Figure 2) suggested that corrosion rates were similar to those observed with THP1 $\pm$ LPS, but less than conditions where dextrose was added. A limitation of the current study was the use of packaged blood from donors that had to be "reconstituted" to simulate freshly drawn blood and therefore the exact composition was unknown. However, microscopic examination showed intact red blood cells and THP1 cells throughout the experiment time. Nevertheless, these results suggest that corrosion of titanium-based implants is different in blood than in more standard corrosion electrolytes.

EIS and cyclic polarization measurements both supported a productive influence of THP1 cells at the implant surface (Figures 3 and 4). The solution resistance of the PBS and media with THP1 cells was is in good agreement with published values for similar solutions.[55] In the cyclic polarization measurements, the linear region observed as current increased suggested that these implant surfaces were highly protected from breakdown. Negative hysteresis observed as the current decreased suggested passivation. These observations are consistent with the low corrosion observed clinically with titanium-based implants.[56] Although some form of coating is suggested from these tests, the β-cathodic Tafel slopes (Table I) indicated that the corrosion mechanism was not oxygen limited.

CONCLUSIONS

The current study suggests that the corrosion risk of titanium-based implants is higher under conditions of biological inflammation and high glucose concentrations.

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